



Predicting ionized hypocalcemia: External validation of an ionized calcium prediction model in patients with COVID-19 and renal failure

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Abstract

Background: Ionized hypocalcemia is common in critically ill patients with COVID-19 and is associated with adverse outcomes. We previously developed a linear model that estimates ionized calcium (I_{Ca}) by adjusting total calcium (T_{Ca}) for the three components of the anion gap and albumin. On internal validation, it outperformed the popular method that corrects T_{Ca} for albumin alone (cT_{Ca}) in diagnosing low I_{Ca} . In this study, we sought to externally validate our I_{Ca} model in hospitalized COVID-19 positive patients.

Methods: We retrospectively studied all 200 patients with COVID-19 who were admitted to the State University of New York Downstate Medical Center between March 11th and April 30th 2020 and referred to the nephrology service for renal failure, and who had I_{Ca} measured on a venous blood gas within 25 min of a comprehensive metabolic panel. We compared the performance of the I_{Ca} model and cT_{Ca} in diagnosing low I_{Ca} by ROC analysis, and also examined the accuracy of the absolute values predicted by the two methods relative to measured I_{Ca} .

Results: On ROC analysis, the I_{Ca} model was better than cT_{Ca} (area under ROC curve: 0.872 [0.025] vs. 0.835 [0.028]; $p = 0.045$). The I_{Ca} model estimated I_{Ca} accurately, but the cT_{Ca} method seemed to overcorrect T_{Ca} , as a substantial number of patients with clearly normal cT_{Ca} values had low I_{Ca} .

Conclusions: In an external validation cohort, the I_{Ca} model estimated I_{Ca} accurately and was better than cT_{Ca} in the diagnosis of low I_{Ca} . This finding can be useful in guiding direct I_{Ca} testing.

Keywords

Calcium, electrolytes

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Introduction

New York City was the epicenter of the COVID-19 pandemic, affecting more than 3400 persons per 100,000 in the borough of Brooklyn alone.¹ Many people were critically ill with multiorgan system involvement and required intensive care. Low ionized calcium (I_{Ca}) is a common abnormality among critically ill COVID-19 patients and has been found to be an important predictor of COVID-19 positivity at triage and of a poor outcome in infected patients, in analyses that accounted for pH and other covariates.²⁻⁴ Although low I_{Ca} can be diagnosed with the ion-selective electrodes

available on blood gas analyzers and other point-of-care analyzers, direct measurement is still not routine, because of its cost, labor and sample requirements,⁵⁻⁸ and the ferocity

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of the pandemic has further strained hospital resources. As a result, it is still common clinical practice to infer the presence of an abnormal I_{Ca} indirectly, using an equation that “corrects” the concentration of total calcium (T_{Ca}) for that of albumin, with the resultant corrected value (cT_{Ca}) compared to the reference range for T_{Ca} .⁹ The cT_{Ca} equation was popularized in the 1970s, without validation against I_{Ca} . Subsequent external validation studies, which incorporated I_{Ca} , found the diagnostic performance of cT_{Ca} to be poor,^{8,10-12} but by then its routine use in clinical practice had been cemented. Commonly suggested reasons for the poor performance of cT_{Ca} include its failure to account for alterations in the affinity of albumin for calcium caused by changes in pH and free fatty acids in critical care patients, or for the differences among centers in the albumin assay.^{8,10-14} Another suggested reason is that cT_{Ca} does not account for variation in the level of small anions that can bind calcium.^{13,15-17}

To account for binding of calcium to small anions and albumin, we recently derived a novel linear regression equation (I_{Ca} model) that estimates I_{Ca} in critical care patients from routine measurements of T_{Ca} , serum albumin, and the three components of the anion gap—sodium (Na), chloride (Cl), and total carbon dioxide (tCO_2).¹³ In that study, the serum chemistry measurements were made using the Siemens ADVIA Chemistry System 1650 and 1800 analyzers, while I_{Ca} was measured using the Radiometer ABL800 FLEX analyzer. The model’s output consists of the most likely arterial I_{Ca} value for a given patient together with a 95% prediction interval (PI), the range that should include the patient’s true value 95% of the time. Upon internal validation, the model significantly outperformed cT_{Ca} in detecting low I_{Ca} . Since external validation is essential to demonstrate a model’s predictive value,¹⁸ in the present study, we tested the ability of the I_{Ca} model to diagnose low I_{Ca} and to estimate the concentration of I_{Ca} at a different clinical site using a different chemistry analyzer in patients who had closely timed chemistry and venous blood gas (VBG) panels during hospitalization for severe COVID-19 infection, and who, in the course of which, were referred to the nephrology service for renal failure.

Methods

Data collection and pairing

In March 2020, the State University of New York (SUNY) Downstate Medical Center was designated as COVID-19-only hospital by state directive.¹⁹ We retrospectively collected serum comprehensive metabolic panel and VBG panel results of every COVID-19 positive patient referred to the nephrology service at our center between March 11th and April 30th 2020, excluding metabolic panels missing T_{Ca} , albumin, Na, Cl, or tCO_2 and VBGs missing I_{Ca} . The diagnoses for which patients were seen by the nephrology service were either acute kidney injury or pre-existing end

stage renal disease (ESRD), with the majority admitted to intensive care. Age and sex were recorded for each patient. In addition, we recorded and paired every metabolic panel with a VBG panel—if the panels were separated by no more than 25 min. Limiting each patient to the single most closely-timed pair of panels, and choosing the earliest pair in case of ties, left 200 pairs, with a mean intra-pair time gap of 0.87 min (range: 0–22 min). The protocol was approved by the institutional review board with a waiver of the need for informed consent (IRB no. 1584306-6).

Analytic methods

Serum metabolic panels were performed on the Beckman AU5800 analyzer (Beckman Coulter, Brea, CA, USA). The T_{Ca} assay uses the Arsenazo III method, with a linear range (LR) of 1.00–4.49 mmol/L (4.0–18.0 mg/dL) and an expected coefficient of variation (%CV) of 1.34% at a T_{Ca} of 2.03 mmol/L (8.12 mg/dL); the local reference range (LRR) is 2.05–2.50 mmol/L (8.2–10.0 mg/dL). Albumin was measured by the bromocresol green method (LR: 15–60 g/L; %CV: 1.5% at 29 g/L; LRR: 35–57 g/L). TCO_2 was measured by an enzymatic method (LR: 2.0–45.0 mmol/L; %CV: 4.0% at 31 mmol/L; LRR: 21–31 mmol/L). Indirect potentiometry was used to measure Na (LR: 50–200 mmol/L; %CV: 0.9% at 127 mmol/L; LRR: 136–145 mmol/L) and Cl (LR: 50–200 mmol/L; %CV: 0.9% at 83 mmol/L; LRR: 98–107 mmol/L). I_{Ca} was obtained from whole blood VBG panels analyzed on the ABL837 FLEX analyzer (Radiometer, Copenhagen), with I_{Ca} measured by calcium electrode (LR: 0.23–3.86 mmol/L; local %CV: 0.4% at 1.03 mmol/L; LRR: 1.15–1.29 mmol/L). VBGs were collected using the PULSATOR sampler (Smiths-Medical, Keene, NH, USA).

Data analysis

Continuous variables are summarized as the mean and SD and compared using the paired *t*-test and simple correlation. Hypocalcemia in VBGs was defined as $I_{Ca} < 1.11$ mmol/L, as in our prior study.¹³ For each patient, the anion gap was calculated as: Na (mmol/L) – Cl (mmol/L) – tCO_2 (mmol/L), and cT_{Ca} (mmol/L) was calculated as: T_{Ca} (mmol/L) + $0.02 \times [40 - \text{albumin (g/L)}]$.⁹ I_{Ca} (mmol/L) was estimated by the I_{Ca} model as: $0.219 + 0.365 \times T_{Ca} - 0.0034 \times \text{albumin} - 0.0042 \times \text{Na} + 0.0073 \times \text{Cl} + 0.0047 \times tCO_2$; the average 95% PI of this estimate is: ± 0.115 mmol/L (i.e., an average range of 0.23 mmol/L).^{13,20}

The I_{Ca} and cT_{Ca} models were examined and compared in two ways. Calibration was assessed by examining their mean predictions relative to the observed mean I_{Ca} values in the entire cohort and in subgroups. Diagnostic discrimination for ionized hypocalcemia was assessed with ROC curves. Since the hypothesis being tested is that the I_{Ca} model would outperform cT_{Ca} in ranking patients for

hypocalcemia, the areas under the curves (AUC) and SE were compared using a one-tailed test in accordance with the method of Hanley and McNeil.²¹ The Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD) guideline was utilized,²² and the checklist is available in the Supplement. IBM SPSS Statistics for Windows, Version 24.0 (Armonk, NY: IBM Corp) was used.

Results

Baseline characteristics and simple comparisons

The characteristics of the cohort and their biochemical measurements are shown in Table 1. The mean value of T_{Ca} (2.06 mmol/L) was just above the lower limit of normal

Table 1. Demographic and laboratory data.

Age (years)	65.7 ± 15.4
Male: Female (n)	124:76
End stage renal disease (n) (%)	81 [40.5%]
Acute kidney injury	119 [59.5%]
T_{Ca} (mmol/L)	2.06 ± 0.20 [1.60–2.75]
I_{Ca} (mmol/L)	1.10 ± 0.10 [0.85–1.39]
Albumin (g/L)	31.6 ± 7.0 [15.2–47.7]
Sodium (mmol/L)	137.4 ± 9.2 [106–175]
Chloride (mmol/L)	100.7 ± 9.8 [76–139]
TCO_2 (mmol/L)	21.6 ± 5.8 [4.00–39.00]
Anion gap, mmol/L	15.0 ± 5.9 [4–44]

Data are presented as mean ± SD [minimum–maximum]. To convert total calcium to mg/dL, divide by 0.2495. To convert albumin to g/dL, divide by 10. Hypercalcemia was infrequent: 9 cases had $I_{Ca} \geq 1.3$ mmol/L.

(LLN) for T_{Ca} (2.05 mmol/L), while the mean of I_{Ca} (1.10 mmol/L) was well below its LLN (1.15 mmol/L; $p < 10^{-10}$). Ionized hypocalcemia, defined here as $I_{Ca} < 1.11$ mmol/L, was present in 96 of 200 patients (48%), and was more common in the patients with ESRD than in the remaining patients (57% versus 42%, $p < 0.05$). Albumin correlated with T_{Ca} ($r = 0.50$ $p < 10^{-13}$), but not with either I_{Ca} ($r = -0.07$ $p = 0.31$) or the anion gap ($r = 0.016$ $p = 0.82$). I_{Ca} tended to decrease as the anion gap increased ($r = -0.21$ $p = 0.003$). The correlation of I_{Ca} with the three “indirect” measures of calcium status was strongest with the I_{Ca} model ($r = 0.77$ $p < 10^{-39}$), intermediate with cT_{Ca} ($r = 0.71$ $p < 10^{-31}$), and weakest with T_{Ca} ($r = 0.58$ $p < 10^{-18}$).

Diagnostic performance and calibration

The AUC of the I_{Ca} model was better than that of cT_{Ca} (0.872 [0.025] versus 0.835 [0.028]); $p = 0.045$; Figure 1) and much better than that of T_{Ca} (0.787 [0.032]). The AUC difference remained significant when we compared novel cT_{Ca} and I_{Ca} models derived and internally validated in the present cohort (see Supplemental Results).

There was reasonable agreement between mean I_{Ca} values predicted by the model and those observed across 6 subgroups (Table 2A), with the model having a slight overall positive bias of +0.022 mmol/L (1.124 [0.082] versus 1.102 [0.099] mmol/L, $p < 10^{-5}$). The imprecision of the model’s predictions was at least as good as expected. For example, of the 37 patients whose predicted I_{Ca} values fell within the 0.03 mmol/L range from 1.08–1.11 mmol/L, the central 95% ($n = 35$) had observed I_{Ca} values that ranged from 0.98–1.16 mmol/L; the observed range (0.18 mmol/L)

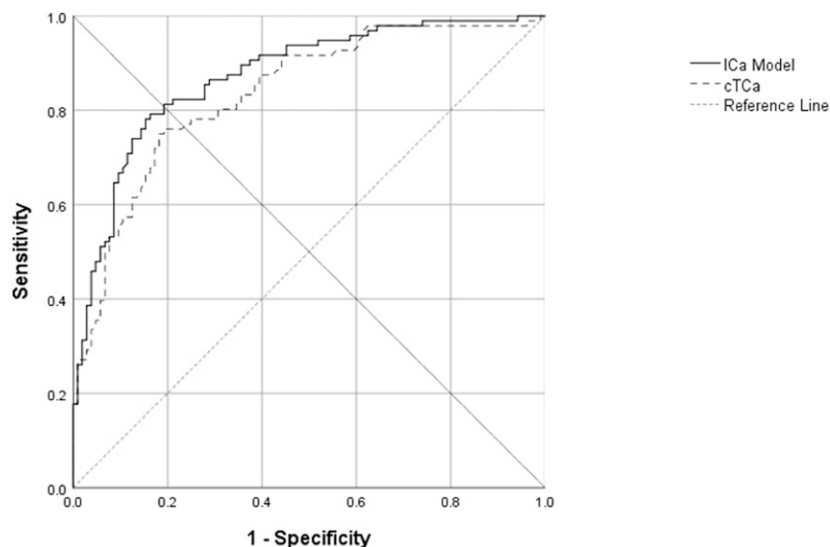


Figure 1. ROC curves illustrating the overall diagnostic performance for hypocalcemia of the I_{Ca} model (solid line) and cT_{Ca} (broken line). The AUCs are 0.872 (0.025) for the I_{Ca} model and 0.835 (0.028) for cT_{Ca} ($p = 0.045$). A diagonal line depicts the points of equal sensitivity and specificity. Going from upper left to lower right, it intersects the I_{Ca} curve at predicted $I_{Ca} < 1.120$ mmol/L (sensitivity and specificity ~81%) and the cT_{Ca} curve at $cT_{Ca} < 2.230$ mmol/L (sensitivity and specificity ~77%).

Table 2. I_{Ca} values and hypocalcemia rates observed across six prediction categories of the I_{Ca} model (A) and of the cT_{Ca} equation (B).

(A) Patients Ranked by I_{Ca} Model				
I_{Ca} model category ^a	N	Mean predicted I_{Ca} ^a	Mean I_{Ca} observed [range] ^a	Rate of low I_{Ca} (%)
<1.0	11	0.95 ^b	0.96 [0.85–1.07]	100
1.00 to <1.10	64	1.06	1.04 [0.89–1.21]	83
1.10 to <1.15	53	1.12	1.10 [1.01–1.18]	43
1.15 to <1.25	60	1.19	1.16 [0.89–1.35]	13
1.25 to <1.30	6	1.27	1.24 [1.08–1.32]	17
≥1.30	6	1.33 ^b	1.30 [1.24–1.39]	0
(B) Patients Ranked by cT_{Ca} Model				
cT_{Ca} category ^a	N ^c		Mean I_{Ca} observed [range] ^a	Rate of low I_{Ca} (%)
1.55 to 1.93	11		0.94 [0.85–1.04]	100
1.94 to <2.19	64		1.05 [0.89–1.17]	77
2.19 to 2.28	53		1.11 [0.89–1.24]	45
2.29 to 2.52	60		1.17 [0.95–1.35]	17
2.53 to 2.56	6		1.24 [1.18–1.30]	0
2.57 to 2.72	6		1.20 [0.94–1.39]	33

^aUnits are mmol/L.

^bMinimum and maximum predicted I_{Ca} were 0.91 and 0.998 mmol/L for first category (<1.0 mmol/L) and 1.30–1.34 mmol/L for the sixth category (≥1.30 mmol/L).

^cThe sizes of the cT_{Ca} categories were chosen to match those used for the I_{Ca} model in section A above.

was better than the range of model's expected 95% PI (0.23 mmol/L).

Table 2B shows the performance of cT_{Ca} with the patients ranked into subgroups identical in size to those of Table 2A. The rates of hypocalcemia observed across the 6 subgroups show, as in the ROC analysis, that ranking by cT_{Ca} was worse than ranking by the I_{Ca} model. A more significant shortcoming of cT_{Ca} , as the overall mean cT_{Ca} of 2.23 (0.18) mmol/L suggests, was its misleading calibration relative to I_{Ca} . For example, the cT_{Ca} values in the third subgroup ranged from 2.19–2.28 mmol/L. Since each value was well above the LLN for T_{Ca} , a negligible risk of hypocalcemia would be expected. However, the mean I_{Ca} in this subgroup was only 1.11 mmol/L, and the rate of hypocalcemia was substantial (45%). This problem is evident in the fourth and sixth cT_{Ca} subgroups too.

Discussion

Abnormal I_{Ca} is detected poorly by cT_{Ca} , which adjusts T_{Ca} for albumin alone.^{8,10–12} Since calcium binds to small anions as well as to albumin, the further adjustment of T_{Ca} for the anion gap has been suggested as a possible improvement.^{13,15–17} We previously derived a model that estimates I_{Ca} by adjusting T_{Ca} for the components of the anion gap and albumin. On internal validation, it was better than cT_{Ca} in the diagnosis of ionized hypocalcemia.¹³ In the present study, we confirmed that I_{Ca} varies inversely with the anion gap, and externally validated our I_{Ca} model in a cohort of hospitalized patients with COVID-19 who

developed concomitant renal injury or had pre-existing renal disease, conditions in which low I_{Ca} is prevalent and of clinical importance.^{2–4,8} On ROC analysis, the I_{Ca} model was better than cT_{Ca} in the relative ranking of patients for the diagnosis of hypocalcemia (Figure 1). This was also evident by comparing the respective hypocalcemia rate trends observed across subgroups ranked by the I_{Ca} model and by the cT_{Ca} equation (Table 2).

In absolute terms, the cT_{Ca} equation appeared to “overcorrect” T_{Ca} by assigning values well above the LLN to several subgroups in Table 3 that proved to have significant rates of ionized hypocalcemia. Since cT_{Ca} values are intended to be judged by the reference range for T_{Ca} , reliance on cT_{Ca} will cause the diagnosis to be entirely overlooked in those patients. This poor alignment of cT_{Ca} with I_{Ca} could be the result of the absence of critically ill patients in the cT_{Ca} equation's original derivation cohort, or calibration differences between the original and the local laboratories, and it could hypothetically be mitigated by introducing a variety of local center corrections, with the simplest being a fixed reduction to cT_{Ca} of, for example, 0.17 mmol/L (equivalent to changing the reference value of albumin used in the cT_{Ca} equation from 40 g/L to the current cohort's mean of 31.6 g/L).^{14,23} It is noteworthy that the I_{Ca} model required no such local correction in this external validation study. It provided accurate absolute estimates of mean I_{Ca} within the cohort overall and its subgroups, with only a small positive bias of approximately 0.02 mmol/L (Table 2). A bias of this size is clinically inconsequential, and especially so in respect to the size of the I_{Ca} model's expected 95% PI (± 0.115 mmol/L), a

degree of imprecision similar to that of a recent canine I_{Ca} model.¹⁷ Such imprecision is inevitable when regression equations are applied to individual patients, with an extreme example being the 95% PI of glomerular filtration rate estimating (eGFR) equations.²⁴⁻²⁶ Consequently, although the I_{Ca} model's estimates are accurate on average, owing to their imprecision, they cannot replace direct I_{Ca} measurement, the gold standard for guiding treatment decisions at the individual patient level. Low I_{Ca} is associated with increased risk in COVID-19, but its measurement is relatively costly. Therefore, we propose that clinicians can more efficiently screen for low I_{Ca} and, in turn, decide when confirmatory I_{Ca} testing is needed by using the I_{Ca} model's point prediction in one of two ways—either by simply comparing it to a cutoff (e.g., <1.15 mmol/L) or by combining it with its 95% PI to define a range (i.e., point prediction \pm 0.115 mmol/L) that can be used to intuitively assess the likelihood of low I_{Ca} .

Although the I_{Ca} model may not be easy to memorize, its output can be feasibly reported in metabolic panel reports, as is common practice for cT_{Ca} , anion gap, eGFR, and other forms of laboratory-based clinical decision support. Alternatively, the model output could be easily obtained with an internet-based calculator or smartphone app (one is available on the qxmd.com website).²⁰

The strength of our study is that it tested a prior hypothesis and that the patients studied had COVID-19 and renal failure, conditions in which identification of low I_{Ca} can have a clinical impact. Nonetheless, since most of these patients were critically ill, the application of the I_{Ca} model to less seriously ill patients, such as outpatients with ESRD, cannot be established from the present study, although the model has performed well in such patients in our informal experience. Further validation on other analytic platforms is also warranted. Other limitations of the study include its retrospective data collection, so the possibility that some VBG samples were processed with an excessive delay, potentially resulting in an artifactual fall in pH and reciprocal rise I_{Ca} ,⁸ cannot be excluded, and that the cohort studied was relatively small and generated by referrals.

In summary, we externally validated our linear I_{Ca} model in a novel laboratory environment by showing that it accurately predicted I_{Ca} and was better than cT_{Ca} in diagnosing ionized hypocalcemia. The point predictions of the model, together with their 95% PI, can be used to help clinicians decide when direct I_{Ca} measurement is needed in individual patients.

Declaration of conflicting interests

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Ethical approval

The protocol was approved by the SUNY Downstate Health Sciences University Institutional Review Board & Privacy Board with a waiver of the need for informed consent (IRB no. 1584306-6).

Guarantor

EY.

Contributorship

EY and PG contributed to the study design, statistical analysis, and results interpretation. YM, IP, and JO contributed to data acquisition. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Supplemental Material

Supplemental material for this article is available online.

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